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Proline treatment improves physiological responses in quinoa plants under drought stress

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Water deficiency is an important abiotic stress that often linked with other major abiotic stresses as heat stress, salinity stress, etc. Thus, it is considered as one of the main factors responsible of reduction crop productivity. Egypt presents a distinctive example of drought problem faced in some arid and semi-arid regions. Thus, water conserving becoming a crucial consideration for agriculture. Two field experiments were conducted during two successive seasons at the Research and Production Station of National Research Centre, Nubaryia district, Beheira Governorate, Egypt to investigate the physiological role of proline with 12.5 & 25mM concentrations in improving growth, some biochemical, osmolytes compounds and yield as well as seed nutritional values and quality of quinoa plant under normal and water deficit conditions. Skipping irrigation two times led to marked decreases in growth criteria, photosynthetic pigments, endogenous indole acetic acid (IAA), yield quantity and quality. Meanwhile, increase phenolics, total soluble sugars, proline and free amino acids contents of quinoa leaves.On the other hand, exogenous application of proline led to marked increases in growth characters (plant height, shoot, root fresh and dry weight) concomitantly with increasing the levels of photosynthetic pigments, IAA, phenolics, free amino acid and proline contents as compared with untreated plants. Moreover, different treatments increased seed yield and its components, also marked increases in nutritional values of the yielded seed (carbohydrate contents, protein%, oil, flavonoids and antioxidant activity). It is noticed that higher concentrations of 25 mM proline was more pronounced than lower concentration in increasing most of the tested parameters of quinoa plant.

Keywords: Antioxidant activity, Growth, Osmolyte, Quinoa, Sandy soil, Proline, Yield.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd) is an original food crop can replenish part of foodstuff gap. It is a food crop recently introduce in Egyptian lands. Because of its high nutritive value seeds can be utilized for human food, in flour production and in animal feedstock (Bhargava et al. 2007). Quinoa could be used in bread in combination or substitution of wheat and other seed products (Shams, 2010). Moreover, quinoa is considered as a multipurpose crop because of the high-quality protein seeds, especially rich in essential amino acids, minerals, carbohydrates, antioxidant compounds as carotenoids, flavonoids, vitamin C and dietary fiber compared to that of cereals such as corn, oat, rice and

wheat (Repo-Carrasco et al. 2011). Quinoa crop was chosen by FAO as one of the important crops which play major role in food security assuring in the 21th century due to its high nutritional value and its good tolerance to adverse climatic conditions (Jacobsen, 2003). It is recommended as useful essential food industries for formulations of baby gluten-free foods (Ogungbenle, 2003). Also, because this crop can grow in sandy soil of arid and semiarid regions so, it is used to replenish part of food gap.

Egypt presents a distinctive example of drought problem faced in some arid and semi-arid regions. Water deficiency which often linked with other major abiotic stress such as heat stress, salinity stress, etc. so, it is considered as one of the primary factors responsible for crop productivity reduction (Ashraf, 2010). Thus, water conserving becoming a crucial consideration for agriculture. Water deficiency caused adverse effect on plants via reduced growth, nutrient attainment reduction and alteration, in water status of plants (Ali & Ashraf, 2011). During photosynthesis, water deficiency induced reduction of photosynthetic efficiency because of increased accumulation of reactive oxygen species (Hasanuzzaman et al. 2014). In addition, the decrease in water contents in cells cause loss of cell enlargement and turgor as well as a decrease in water potential of leaf all of these caused water deficit or drought stress. The metabolism disturbance, photosynthesis decline and finally the death of the plant are the result of high water stress (Dawood & Sadak 2014). In addition, the response of plants to water stress vary significantly depending on the duration and intensity of stress as well as species of plant and the growth stage of plant (Dacosta & Huang, 2007). A common response of plant to drought, salinity, low and high temperature stresses is by accumulation of some compatible osmolytes or solutes as soluble sugars, amino acids as proline (Sadak et al. 2010; Abdelhamid et al. 2013). These compounds acts as osmoprotectants and stabilize biomolecules of cells under stress conditions (Theerakulpisut & Phongngarm, 2013). Ranganayakulu et al. (2015) stated that, water stress resulted in a significant accumulation of free proline content in leaves of groundnut cultivars. Therefore, development of methods and strategies to alleviate the harmful effects of drought stress in plants is necessary and received considerable attention. Stress tolerance enhancement in plants has major implications in agriculture and horticulture (Senaratna et al. 2000). Stress tolerance is a complex trait that is controlled by multiple genes and involves different physiological and biochemical mechanisms (Zhang & Shi, 2013). Osmoprotectants using as foliar treatment or seed priming can be an economically viable strategy for improving tolerance of stress under adverse environmental conditions (Abdelhamid et al. 2013; Sadak & Mostafa, 2015; Sadak, 2016). One of this these osmolytes is proline which induced by stress in plants. Accumulation of proline in plant tissue exposed to stress have several functions as: osmoprotection (Kishor et al. 1995), anti-oxidation (Hoque et al. 2007), reserve of C and N for growth after stress relief (Hayashi et al. 2000), proteins and membranes stabilization and macromolecules protection from denaturation (Hamilton and Heckathorn, 2001), and as readily available source of energy and reducing power (Stewart et al. 1974). The effect of proline is dependent on its concentration because an excessive amount of free proline has negative or side effectson cell growth or on protein functions (Abdelhamid et al. 2016). The effectiveness of proline applied as a foliar spray depends on plant developmental stage, species type, application time and concentration (Ashraf & Foolad, 2007). Therefore, it is necessary to determine the optimal concentrations of exogenously applied proline that can provide beneficial effects in new crop plants, such as quinoa, when exposed to abiotic stress.

So, this investigation was conducted to study the physiological role of exogenous applications of proline on various growth criteria, some biochemical and physiological constituents as photosynthetic pigments, indole acetic acid, phenol, total soluble sugars, proline and free amino acids, yield and some biochemical compounds of the yielded seeds ofquinoa plants grown under drought. The aim was to improve our understanding of the mechanisms of drought stress tolerance in proline-treated plants.

MATERIALS AND METHODS

Plant material and growth conditions

A field experiment was conducted at the Research and Production Station of National Research Centre, Nubaryia district, Beheira Governorate, Egypt, during two successive seasons of 2014/2015 and 2015/2016. The soil of both experimental sites was newly reclaimed sandy soil where physical and chemical analysis

is reported in Table 1 according to (Chapman & Pratt, 1978).

Table 1: Physical and chemical analysis of the experimental soil sites.

Mechanical analysis:	
Sand	
Course 2000-200µ%	47.64
Clay < 2µ% Fine 200-20µ %	36.59
Silt 20-0µ%	12.66
Clay < 2µ%	4.18
Soil texture	Sandy
Chemical analysis:	
pH 1:2.5	7.5
EC dSm^{-1}	0.13
$CaCO_3$	5.3
OM%	0.06
Soluble cations (meq/l)	
Na^+	0.59
K^+	0.14
Mg^+	0.95
Ca^{++}	1.0
Soluble anions (meq/l)	
CO_3^{--}	0.00
HCO_3^-	1.27
Cl^-	0.46
SO_4^{--}	0.87
Macro elements (ppm)	
N	51
P	12.2
K	74
Micro elements (ppm)	
Zn	0.13
Fe	1.03
Mn	0.28
Cu	0.00

Seeds of quinoa cultivar (*Chenopodium quinoa* Willd.) cv. Quinoa 1 were obtained from Agricultural Research Centre, Giza, Egypt. The experimental design was split – plot with four replications. The main plots were devoted to the drought stress treatments, while the different concentrations of proline (0, 12.5 mM and 25mM) were randomly occupied the sub – plots. Quinoa seeds were sown on 20 October in both seasons at the rate of 3 kg/faddan (one faddan =0.42 ha) in rows 3.5 meters long, and the distance between rows was 20 cm apart. Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The recommended agricultural practices of growing quinoa were applied. Pre-sowing, 150 kg/faddan of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% N at a rate of 75 Kg/faddan in five equal doses before the 1^{st}, 2^{nd} ,3^{rd}, 4^{th} and 5^{th} irrigation. Potassium sulfate (48-52 % K_2O) was added in two equal doses of 50 kg/faddan, before the 1^{st} and 3^{rd} irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Proline foliar treatment consisted of three levels of proline namely 0 mM (control), 12.5mM and 25.0 mM considered as Pro 0, Pro 1 and Pro 2, respectively. Drought stress including normal irrigation (D0) and skipping two irrigation times at 45 and 52 days after sowing (D1). Different proline concentrations were applied twice; where plants were sprayed after 30 and 34 days from sowing. Plant samples were taken after 60 days from sowing for estimation some growth parameters such as plant height (cm), branches & leaves number/plant, fresh & dry weights of shoot/plant (g), relative water content% (RWC), root length (cm), fresh & dry weights of root/plant (g), photosynthetic pigments in fresh leaves, endogenous indole acetic acid (IAA), phenolics and total soluble sugars, Oven- dried leaves (for 72 h at 70 ºC) were ground to a powder and kept in a desiccators to determine proline and free amino acids. Yield and its components as plant height (cm), number of fruiting branches/plant, dry weight of plant (g), seed weight/plant and 1000 seeds weight (g), as well as nutritive value of the yielded seeds as total carbohydrates%, protein%, flavonoids content, phenolic contents, oil%, phosphorus and potassium contents, in addition to antioxidant activities %.

Biochemical analysis

Chlorophyll a, chlorophyll b and carotenoids concentrations were estimated using the method of Moran (1982). Indole acetic acid was determined according to the method reported by Larsen et al. (1962). Phenolics contents were determined using the method described by Danil & George (1972). Total soluble sugars were extracted by using the method of Homme et al. (1992). TSS was analyzed by using Spekol Spectrocololourimeter VEB Carl Zeiss (Yemm and Willis, 1954). Proline and free amino acids were extracted according to the method described by Vartanain et al. (1992). Proline was assayed according to the method described by Bates et al. (1973). Free amino acid content was determined with the ninhydrin reagent method (Yemm&

Cocking, 1955). Total carbohydrates were determined according to Dubois et al. (1956). The protein content was determined by micro-kjeldahl method according to AOAC (1990). Seed oil content was determined using Soxhlet apparatus and petroleum ether (40-60^{o}C) according to AOAC (1990). Total flavonoid contents were measured by the aluminum chloride colorimetric assay as described by Ordoñez et al. (2006). Free radical scavenging activity was determined according to Brand- Williams et al. (1995) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent.

Statistical analysis

The analysis of variance procedure of split-plot design according to Snedecor & Cochran (1990), treatments means were compared using Duncan (1955) test at 5% of probability and presented with the standard errors. Combined analysis of the two growing seasons was carried out according to (Steel &Torrie, 1960).

RESULTS AND DISCUSSION

Water is one of the most important factors effect on crop production, affecting not only agricultural output but also food security (Garg et al. 2002). Increasing plant tolerance to abiotic stress especially drought stress is a major goal for both farmers and scientists. Proline as an osmo protectant could alleviate the reduced effect of drought stress via its effect on cellular osmotic potential.

Growth criteria

Data presented in Table 2 A & B show the effect of proline foliar treatment with different concentrations on quinoa plant under drought stress. Skipping irrigation at 45 and 52 days (Drought stress) caused significant decreases in quinoa plant growth criteria (shoot length, branches and leaves number/plant, fresh & dry weights of shoot/plant, RWC% of shoot). Meanwhile, increased root parameters (length, fresh and dry weights of root/plant) relative to control plants (D0 Pro0) (Table 2 A & B). In agreement with these results, Abdelhamid et al. (2016) stated that both fresh and dry weights of shoots of fenugreek decreased with decreasing WHC and referred these decreases to disorders induced by stress and generation of reactive oxygen species (ROS). In addition, these decreases in shoot height in response to drought stress might be due to decrease in cell elongation, cell turgor, cell volume and eventually cell growth (Banon et al. 2006). Moreover, drought affects plant–water relations, decreases shoot water contents, causes osmotic stress, inhibits cell expansion and cell division as well as growth of plants as a whole (Alam et al. 2014). Meanwhile, the increases in root length, fresh & dry weight of quinoa plant because of the initial effects of drought stress (Table 2B), quinoa plants started to divert assimilates from stem and utilized them for increased root growth in order to increase water absorption (Abdelhamid et al. 2016). On the other hand, proline treatments proved to be effective in enhancing shoot & root length, fresh and dry weights of shoot and root under unstressed and drought stressed plants (Table 2 A & B). It was noted that Pro2 was more effective than Pro1 treatment at unstressed and drought stressed conditions as it caused significant increases in dry weight of shoot by 94.32% and 109.77%, respectively, compared to 51.22% and 27.57% at Pro1. It was interested to observe a considerable increment in root length of proline-treated plants more than the control and the drought-stressed plants. Fresh and dry mass of roots were also increased in response to proline treatment under stress and un-stress conditions (Table 2 A & B). Similar findings were observed previously by Pro treatment with different plants (Sadak & Mostafa, 2015; Abd Elhamid et al. 2016; El- Awadi et al. 2016). The mitigation effect of proline on adverse effects of drought on growth may be via improving water status of plant tissues, since the relative water content of the shoot increased (Table 2A). (Sadak & Mostafa, 2015) indicated that proline exogenous treatments on sunflower plants improve water retention and plant tolerance through osmoregulation and stomatal closing at stress. The increases in growth characters caused by different proline concentrations especially at 25 mM might be due to the role of proline in protecting enzymes, 3D structures of proteins and organelle membranes and also supplies energy for growth and survival thereby helping the plant to tolerate stress (Hoque et al. 2007). As well as, proline as osmoprotectant might have been absorbed by the developing seedlings, where it increased the influx of water and reducing the efflux of water under drought stress thus it maintained water status of plant (Chen & Murata, 2008) thus increased growth and yield of quinoa plants.

Photosynthetic pigments

The effect of drought stress (skipping

irrigation two times at 45 & 52 days after sowing) and proline foliar application with different concentrations (12.5 & 25 mM) on photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) of quinoa plant are shown in Table 3. Drought stress significantly decreased chlorophyll a, chlorophyll b, carotenoids and consequently total pigments. The percentage of decreases reaches to 7.53%, 28%, 37.5% and 17.96% in chlorophyll a, chlorophyll b, carotenoids and total pigments, respectively, as compared with normal irrigated plants. This reduced effect on photosynthesis was attributed to pigments oxidation that damage photosynthetic pigments and impaired biosynthesis of pigments (Anjum et al. 2011; Pandey et al. 2012). Dawood & Sadak (2014) stated that one symptom of water stress in leaves is loss of chlorophyll indicating some form of disruption of chloroplasts. Meanwhile, proline foliar application significantly increased chlorophyll a chlorophyll b, total carotenoids and consequently total pigments. The maximum increases of the photosynthetic pigments were obtained by foliar application with (25 mM) under normal and water deficit conditions. These increases were 62.37%, 30%, 54.17% and 51.49% in plants treated with higher concentration (25 mM) proline compared to 37.63%, 14.00%, 33.33% and 29.94% in plants treated with lower concentration (12.5 mM) proline for chlorophyll a, chlorophyll b, carotenoids and total pigments. These results were confirmed by the findings of Sadak & Mostafa (2015); Abdelhamid et al. (2016). In this connection, Yan et al. (2011) mentioned that proline foliar treatment not only functioned as a nutrient but also possessed some defensive mechanisms for damaged plants under salt stress. These mechanisms were, promoting photosynthesis, maintaining enzyme activity and scavenging ROS. Ali et al. (2007) explained the beneficial effect of proline applied was due to its promotive effects on photosynthetic capacity by overcoming stomata limitations, enhancing biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from water stress-induced degradation.

Endogenous indole acetic acid (IAA)

The presented data in Table 4 show the variation of endogenous growth regulator (indole acetic acid, IAA) in response to foliar application of quinoa plant with different concentrations of proline subjected under water deficit conditions. Drought stress by skipping irrigation two times induced significant decrease in endogenous IAA, as compared with control plants. This percentage of decrease was 68.76% as compared with control plants. These decreases could be attributed to the effect of water deficit on soil dryness as it increased and soil water potential becomes more negative. So, quinoa plants activate its defense system for absorbing more water (Abdelhamid et al. 2016). Meanwhile, foliar spraying of quinoa plants with proline different concentrations increased significantly IAA contents; the most effective treatment was 25 mM as compared with the corresponding control. The promotive effect of proline under drought stress is more effective than its effect under normal conditions as it increased endogenous IAA content by 59.58% and 120.31% under drought stress compared with 30.95% and 72.61% at normal conditions at Pro 1 and Pro2, respectively. These increases are concomitant with the increases in growth rate of quinoa leaves (Table 2) indicating the physiological role of IAA in increasing growth via stimulating cell division and/or cell enlargement (Taize & Zeiger, 2006).

Phenolics content

Table 4 clearly shows that, skipping irrigation two times (at 45 and 52 days) increased significantly phenolics content of the quinoa plant as compared to control plants. These obtained results are coinciding with those obtained by Lanovici (2011). Abdelhamid et al. (2016) reported that drought stress increased total phenols contents of tomato cultivars and fenugreek plant. These increases in phenolic contents in response to drought stress of quinoa plant might be due to this metabolites (phenolics) may participate in scavenging of reactive oxygen species (ROS) mainly via the antioxidative enzymes utilizing poly phenols as co-substrates (Sgherri et al. 2003). Moreover, proline foliar application to quinoa plant caused more significant increases of phenolic contents of quinoa as compared with untreated controls either at normal irrigated or drought stressed plants. Increase proline concentrations caused a gradual increase of phenolics content as compared with the corresponding control plants. Higher concentration of proline (25 mM) increased phenolics content by 65.75% and 33.33% compared to 34.25% and 20.60% at lower concentration (12.5 mM) with normal and stressed conditions. This effect of proline treatment on quinoa plant, generally, may be due to proline treatment appeared to be effective in

counteracting the negative effects of water stress on phenolic contents. Beside, phenolic the non-photosynthetic pigments investigated in the present study may contribute to the antioxidant activity of wheat plants (Aldesuquy & Ghanem, 2015).

Total soluble sugars

Total soluble sugars (TSS) contents of quinoa leaves in response to treatment of quinoa plants with different concentrations of proline under drought stress are presented in Table 4. Data clearly show that, skipping irrigation at 45 & 52 days after increased significantly total soluble sugars contents of quinoa leaves, this increase reached to 22.09%, as compared with those irrigated normally (control plants). These accumulation of high soluble sugar levels have also been demonstrated in shoots of different plant species under drought stress conditions and foliar treatment with proline different concentrations (Bakry et al. 2012; Dawood & Sadak, 2014; Sadak & Mostafa, 2015; Abdelhamid et al. 2016). These increases of soluble sugars are a response of plants to drought stress has been widely reported (Murakeozy et al. 2003). In addition soluble sugars may act as ROS scavengers so improve membrane stabilization (Hosseini et al. 2014). More accumulation of the total soluble sugars in leaves of the proline-treated plants in both normal irrigated and drought stressed quinoa plants (Table 4). Increasing proline concentration caused a gradual increase in TSS contents; it increased TSS by 12.44% &7.36% in plants treated with lower concentration (Pro l) and 31.84% & 34.88% in plants treated with higher concentration (Pro 2) under normal and drought stress conditions, respectively. These increases may help in turgor up keeping and cellular membrane stabilization (Hosseini et al. 2014).

Proline and free amino acids contents

Data of proline foliar application effect on proline and free amino acids contents of quinoa plants subjected to water deficit are presented in Table 4. Data clearly show that water deficit by skipping irrigation two times at 45 and 52 days after sowing caused significant increases in proline and free amino acid contents, foliar application with different concentrations of proline caused more significant increases of proline and free amino acids contents as compared with the corresponding control. The most effective treatment was 25 mM proline especially at drought stress. Accumulation of compatible osmolytes causing osmotic adjustment in plants under drought stress (Jagesh et al. 2010). Proline has vital roles in osmotic adjustment (Hasegawa et al. 2000), stabilization and protection of enzymes, proteins and membranes (Ashraf &Foolad, 2007) from damaging effects of drought-osmotic stresses. Also, reducing oxidation of lipid membranes (Demiral & Türkan, 2004). Proline addition with drought stress induced proline and free amino acids levels in quinoa plant (Table 4).

Yield and yield components

It is worthy to mention that water availability to plant in different growth stages affect on plant yield and biochemical constituents of the plant and the yielded seeds. Data presented in Table 5 show the effect of skipping irrigation two times (drought stress) decreased yield components (number of pods/plant, weight of pods/plant, weight of seeds/plant and seed index) of quinoa plant. The percentages of decreases in seeds yield/plant and 1000 seeds weight were 66.94% and 7.89%, respectively, as compared with unstressed control. These reductions in yield of quinoa plant are mainly due to the reduction in growth parameters (Table 2) and photosynthetic pigments (Table 3). Meanwhile, spraying quinoa plant with different concentrations of proline (12.5 & 25 mM) could alleviate the adverse effects of drought stress as well as increased in yield and yield components of those plants irrigated unstressed quinoa plants compared with the corresponding water level. The most effective treatment was 25 mM of proline under unstressed and drought stressed conditions. Recently, exogenous protectants such as osmoprotectant (proline, glycinebetaine, trehalose, etc.) have been found effective in mitigating the stress induced damage effect in plant cells (Hoque et al. 2007; Hasanuzzaman et al. 2013).

Nutritional values of the yielded seeds

Data presented in Table 6 clearly show the effect of drought stress and foliar application with different concentrations of proline on some nutritional values of the yielded seeds of quinoa plant (carbohydrates%, protein%, oil%, phosphorous, potassium and nitrogen contents). Skipping irrigation two times significantly decreased the nutritional values of the yielded seeds (carbohydrates%, protein%, phosphorous, potassium and nitrogen contents). In the

Table 2: Effect of different concentrations of proline (12.5 mM and 25 mM) on morphological criteria of quinoa plants subjected to drought stress (means of two seasons).

A: Shoot characters

Drought	Proline conc. (mM)	Shoot length (cm)	No. of branches /plant	No. of leaves/plant	Fresh weight/plant (g)	Dry weight/plant (g)	RWC%
D0	Pro0	36.3^{bc}±0.25	2.09^{c}±0.023	22.3^{d}±0.42	38.7^{d}±0.53	7.75^{d}±0.057	80.15^{c}±0.84
	Pro1	38.3^{b}±0.24	3.08^{b}±0.024	28.3^{b}±0.043	58.6^{b}±0.34	11.72^{b}±0.057	83.05^{b}±0.78
	Pro2	45.3^{a}±0.26	4.07^{a}±0.023	35.0^{a}±.0.34	75.3^{a}±0.46	15.06^{a}±0.074	86.81a±0.78
D1	Pro0	26.3^{e}±0.27	1.06^{d}±0.021	19.3^{e}±0.37	19.9^{f}±0.32	3.99^{f}±0.024	65.51^{e}±0.54
	Pro1	31.3^{d}±0.21	2.09^{c}±0.020	21.3^{d}±0.34	25.4^{e}±0.30	5.09^{e}±0.024	77.34^{d}±0.65
	Pro2	35.7^{c}±0.21	3.09^{b}±0.024	26.3^{c}±0.32	41.8^{c}±0.24	8.37^{c}±0.023	82.55^{b}±0.45

Each value represents the mean ± standard error (n =3).

B. Root characters

Drought	Proline conc. (mM)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
D0	Pro0	15.33^{c}±0.048	1.59^{c}±0.019	0.37^{b}±0.0041
	Pro1	16.33^{bc}±0.054	1.72^{bc}±0.021	0.48^{ab}±0.0021
	Pro2	16.67^{bc}±0.045	2.44^{bc}±0.024	0.41^{b}±0.0033
D1	Pro0	18.25^{b}±0.054	2.27^{bc}±0.020	0.47^{ab}±0.0024
	Pro1	21.33^{a}±0.062	2.53^{ab}±0.024	0.51^{ab}±0.0024
	Pro2	22.33^{a}±0.065	3.30^{a}±0.012	0.58^{a}±0.0035

Each value represents the mean ± standard error (n =3).

Table 3: Effect of different concentrations of proline (Pro1 12.5 mM and Peo2 25 mM) on photosynthetic pigments (mg/g fresh wt) of quinoa plants subjected under drought stress (means of two seasons).

Drought	Proline conc. (mM)	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
D0	Pro0	0.93^{e}±0.005	0.50^{c}±0.0003	0.24^{c}±0.0002	1.67^{c}±0.005
	Pro1	1.28^{c}±0.005	0.57^{b}±0.0003	0.32^{b}±0.0002	2.17^{b}±0.006
	Pro2	1.51 a±0.006	0.65^{a}±0.0003	0.37^{a}±0.0003	2.53^{a}±0.006
D1	Pro0	0.86^{f}±0.006	0.36^{d}±0.0002	0.15^{e}±0.0003	1.37^{d}±0.005
	Pro1	1.18^{d}±0.006	0.49^{c}±0.0003	0.18^{d}±0.0002	1.85^{c}±0.006
	Pro2	1.49^{b}±0.006	0.57^{b}±0.0003	0.16^{d}±0.0003	2.22^{b}±0.006

Each value represents the mean ± standard error (n =3).

Table 4: Effect of different concentrations of proline on IAA (µg/g FW), phenolics (mg/100 g FW), TSS, free amino acids and proline contents (mg/g fresh wt) of quinoa plants subjected to drought stress (means of two seasons).

Drought	Proline conc. (mM)	IAA	Phenolics	TSS	Proline	Free amino acids
D0	Pro0	25.01^{c}±0.562	255.75^{d}±6.352	2.01^{d}±0.005	23.58^{e}±0.324	217.65^{f}±8.752
	Pro1	32.75^{b}±0.652	343.34^{c}±7.254	2.26^{c}±0.005	33.32^{d}±0.387	262.65^{d}±9.241
	Pro2	43.17^{a}±0.687	423.91^{b}±7.325	2.65^{b}±0.005	40.96^{c}±0.410±	298.28^{c}±10.325
D1	Pro0	14.82^{e}±0.321	349.50^{c}±8.356	2.58^{b}±0.006	32.27^{d}±0.432	240.32^{e}±8.356
	Pro1	23.65^{d}±0.485	421.50^{b}±7.523	2.77^{b}±0.007	47.23^{b}±0.473	320.86^{b}±11.325
	Pro2	32.65^{b}±0.542	466.00^{a}±8.965	3.48^{a}±0.005	51.28^{a}±0.514	344.94^{a}±10.658

Table 5: Effect of different concentrations of proline (Pro 1 12.5 mM and Pro 2 25 mM) on yield and yield components of quinoa plants subjected to drought stress (means of two seasons)..

Drought	Proline conc. (mM)	Shoot length (cm)	Number of fruiting branches/plant	Dry weight/plant (g)	Seed yield/plant (g)	1000 seeds weight (g)
D0	Pro0	39.00^{c}±0.425	20.00^{ab}±0.214	21.82^{c}±0.242	6.23^{e}±0.402	1.05^{e}±0.025
	Pro1	46.00^{b}±0.521	20.67^{ab}±0.234	26.63^{b}±0.245	7.96^{d}±0.412	1.45^{d}±0.018
	Pro2	58.00^{a}±0.598	22.50^{a}±0.247	32.04^{a}±0.349	8.56^{bc}±0.426	1.83^{c}±0.020
D1	Pro0	27.00^{d}±0.421	17.00^{c}±0.249	18.44^{d}±0.156	8.72^{bc}±0.412	1.38^{d}±0.018
	Pro1	37.33^{c}±0.475	20.00^{ab}±0.234	22.75^{c}±0.256	10.95^{b}±0.485	1.95^{b}±0.023
	Pro2	39.00^{c}±0.512	18.67^{bc}±0.274	28.97^{b}±0.251	15.01^{a}±0.475	2.45^{a}±0.021

Each value represents the mean ± standard error (n =3)

Table 6: Effect of different concentrations of proline (Pro1 12.5 mM and Peo2 25 mM) on carbohydrate%, protein%, oil% and phosphorus and potassium contents (mg/100 g DW) of quinoa seeds subjected to drought stress (means of two seasons).

Drought	Proline conc. (mM)	Carbohydrate %	Protein %	Oil %	P	K
D0	Pro0	62.02^{c}±1.0523	14.02^{d}±0.514	6.23^{e}±0.042	240.35^{e}±6.235	354.30^{d}±7.524
	Pro1	63.14^{b}±1.325	14.72^{bc}±0.321	6.75^{cd}±0.042	273.97^{c}±6.845	399.63^{b}±7.847
	Pro2	64.90^{a}±1.425	15.70^{a}±0.214	6.99^{c}±0.051	340.97^{a}±6.845	433.63^{a}±8.475
D1	Pro0	60.64^{d}±1.124	13.35^{e}±0.412	6.63^{d}±0.057	220.40^{f}±7.521	319.99^{e}±6.514
	Pro1	61.55^{c}±1.242	14.52^{c}±0.472	7.33^{b}±0.054	255.60^{d}±7.542	350.63d±7.514
	Pro2	63.33^{b}±1.132	15.11^{b}±0.541	7.98^{a}±0.045	324.97^{b}±7.752	386.07^{c}±8.475

Each value represents the mean ± standard error (n =3).

Table 7: Effect of different concentrations of proline (Pro1 12.5 mM and Peo2 25 mM) on flavonoids%, phenolics content (mg/100 g DW) and DPPH% (at 50 and 100 µg/ml)of quinoa seeds subjected to drought stress (means of two seasons).

Drought	Proline conc. (mM)	Flavonoids	Phenolics	DPPH%	
				50	100
D0	Pro0	61.50^{d}±3.954	139.32^{e}±2.751	29.80^{d}±2.142	44.15^{d}±2.485
	Pro1	63.92^{c}±3.0214	159.00^{d}±2.842	33.80^{b}±2.142	48.83^{b}±2.965
	Pro2	66.30^{b}±4.425	189.78^{b}±2.421	36.95^{a}±2.751	51.85^{a}±3.214
D1	Pro0	62.39^{cd}±4.254	147.60^{e}±2.475	27.67^{e}±1.952	43.19^{e}±3.041
	Pro1	65.68^{b}±3.425	170.22^{c}±2.953	31.75^{c}±1.952	45.70^{c}±3.000
	Pro2	69.12^{a}±4.475	205.62^{a}±2.415	34.17^{b}±2.126	48.81^{b}±3.103

Each value represents the mean ± standard error (n =3).

meantime increased significantly oil% of the yielded seeds as compared with unstressed plants. Among nutritional value of the yielded seeds, carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, photo assimilates translocation, and respiration (Sadak et al. 2010). These decreases by drought stress through decreased chlorophyll contents in leaves that caused inhibition of photosynthetic activity, thus it leads to less accumulation of carbohydrates in mature leaves and consequently may decrease the rate of transport of carbohydrates from leaves to the developing seeds (Anjum et al. 2003). Also, the decreases in seed chemical composition might be due to the reduction in many enzyme activities caused by low water supply during the life of plant which lead to metabolic activities changes that result in altered in translocation of assimilates to seeds (Ali et al. 2010). Meanwhile, proline treatments enhanced the nutritional values of the yielded seeds as it increased significantly the above mentioned parameters as compared with the corresponding untreated controls. Increasing proline concentrations from 12.5 to 25mM increased gradually the studied parameters. This promotive effect might be due to the role of proline in protecting enzymes, 3D structures of proteins and membranes of different cell organelles and serves as energy supplies for growth and increasing plant tolerance against abiotic stress (Ashraf & Foolad, 2007). These results are in agreement with those obtained by Sadak & Mostafa (2015) on sunflower; Abdelhamid et al. (2016) on fenugreek plants.

Flavonoids and phenolics contents of the yielded seeds

Table 7 clearly shows that skipping irrigation two times (drought stress) increased markedly flavonoids and phenolics contents of the yielded seeds of quinoa plant. Flavonoids are one of the largest classes of plant phenolics performing different functions in plant system, including pigmentation and defense (Harborne & Williams, 2000).In accordance to our results obtained in the present study, Haghighi et al. (2012) recorded that water stress enhanced the accumulation of flavonoids in *Planta goovata* plants. Moreover, they postulated that this increment in flavonoids content might be due to the induction in enzymatic activity occurring under stress, thereby favoring the production of different flavonoids compounds. Flavonoids and phenolics are nonstructural carbohydrates that tend to accumulate under stress conditions and thus trigger the synthesis of carbon-based defensive substances. In accordance with these results, drought brought about marked increase in the total amount of phenolic compounds in pea and wheat plants as indicated by (Alexieva et al. 2001). Moreover, proline foliar application to quinoa plant caused more significant increases of flavonoids and phenolic contents of the yielded seeds as compared with untreated controls either at normal irrigated or drought stressed plants. Increasing proline concentrations caused gradual increases of flavonoids and phenolics content as compared with the corresponding controls. Generally, proline

treatment appeared to be effective treatment in counteracting the negative effects of water stress on total phenols and flavonoids contents. Beside, phenolic the non-photosynthetic pigments investigated in the present study may contribute to the antioxidant activity of wheat plants (Aldesuquy & Ghanem, 2015).

Antioxidant activity

The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, a measure of seed total antioxidant activity, expressed the percentage reduction of the initial DPPH absorption by the tested antioxidants. Skipping irrigation times two times (water deficit) decreased significantly (DPPH) radical scavenging activities at 50 and 100µg/ml in quinoa seed methanolic extract. On the other hand, exogenous application of proline increased significantly quinoa seed antioxidant activities under stress and non-stress conditions, respectively as compared with untreated plants (Table 7). In the present study, seed antioxidant activities in methanolic extract of quinoa seeds was positively related to seed phenolics, flavonoids, and oil contents (Table 7). The strong positive correlation between total phenolics and antioxidant activity as observed in the present study had already been observed in cereals (Dykes et al. 2007) and soybean (Kumar et al. 2009), which suggests that this increase in seed antioxidant activity is contributed by the presence of high amount of phenolic compounds.

CONCLUSION

It can be concluded that proline foliar treatments (12.5 mM and 25.0 mM) improved growth parameters, relative water content, photosynthetic pigments, indole acetic acid, phenolics, TSS, proline and free amino acids in leaf tissues of quinoaplants. Also, yield, yield components and the nutritional values of the yielded seeds were improved in plants subjected under drought stress conditions by skipping two irrigation times.

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